REMARKS

Prior to a first Office Action in this application, Applicants request that original claims 1-7 be cancelled and new claims 8-30 be added. These new claims do not introduce new matter. Support for new claims 8-30 can be found as follows:

New claim 8 is supported by the specification as originally filed at page 11, lines 17-23, at page 12, lines 24-26, and page 18, lines 4-6.

New claims 9 and 10 are supported by the specification as originally filed at page 16, lines 5-6, and at page 48, lines 7-11.

New claims 11 and 12 are supported by the specification as originally filed at page 11, lines 17-19, and at page 12, lines 1-3.

New claim 13 is supported by the specification as originally filed at page 18, lines 1-2.

New claim 14 is supported by the specification as originally filed at page 15, lines 24-25.

New claim 15 is supported by the specification as originally filed at page 25, line 14, and at page 31, line 8.

New claims 16-18 are supported by the specification as originally filed at page 25, line 23, to page 26, line 2.

New claims 19-21 are supported by the specification as originally filed at page 12, lines 1-3.

New claim 22 is supported by the specification as originally filed at page 12, lines 13-15.

New claim 23 is supported by the specification as originally filed at page 24, lines 9-10.

New claim 24 is supported by the specification as originally filed at page 11, line 8.

New claim 25 is supported by the specification as originally filed at page 19, lines 21-22.

New claim 26 is supported by originally-filed claim 1.

New claims 27-29 are supported by the specification as originally filed at page 16, lines 12-27.

New claim 30 is supported by the specification as originally filed at page 11, lines 17-23.

In addition, Applicants request that the application be amended to enter the Sequence Listing submitted herewith. A Statement Regarding Sequence Listing Under 37 CFR § 1.821(f) is submitted herewith. Applicants have amended the specification as indicated above to introduce SEQ ID NOs, and also to correct obvious typographical errors. Entry of these amendments is respectfully requested.

It is submitted that this application is now in good order for allowance and such allowance is respectfully solicited. Should the Examiner believe minor matters still remain that can be resolved in a telephone interview, the Examiner is urged to call Applicants' undersigned attorney.

Respectfully submitted,

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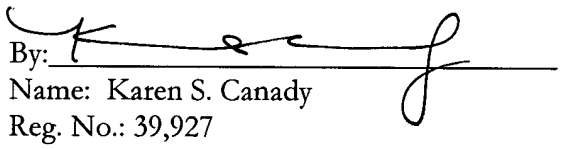
By: 
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APPENDIX: PARAGRAPHS IN MARKED-UP FORM

Please replace the paragraph at page 4, lines 1-8, with the following:

Recombination at the mRNA level may take place during reverse transcription when both packaging mRNA and transfer vector mRNA (even when generated by separated expression constructs) become co-encapsidated into viral particles. The retroviral enzyme reverse transcriptase (RT) uses mRNA as template for DNA synthesis. Also, RT is known to switch between or away templates. Thus, if two different mRNA's are present within a viral particle, when combined, a single DNA unit could be synthesized by the RT as the result of template switching.

Please replace the paragraph at page 19, lines 10-19, with the following:

Furthermore, an HIV-1 RNA element present in the packaging vector gag-pol mRNA was observed to lead to specific encapsidation of significant amounts of the message into released vector particles under certain conditions. The element serves as the HIV-1 major splice donor site (SD) and consists of at least nucleotides, GACUGGUGAG (SEQ ID NO: 1). In the absence of transfer vector expression, vector particles generated only by pMDLg/pRRE packaging construct have no detectable gag-pol RNA message. Analysis of total RNA extracted from the cells which produced the vector particles, showed that expression levels in all cases were similar. When 5'mRNA regions of the tested packaging vectors were compared, it became apparent that the specified above sequence is the determinant which provides specific encapsidation of the messages.

Please replace the paragraph at page 33, lines 5-10, with the following:

The lentiviral packaging plasmids were derived from the plasmid pCMVΔR8.9 (ΔVprΔVifΔVpuΔNef) described previously in Zufferey et al., supra. All the remaining sequences of the nef gene in pCMVΔR8.9 were removed by digesting with XhoI and BstEII, filling in with Klenow and religating. The construction deleted 100 basepairs, joining the

truncated env reading frame of HIV-1 to the genomic insulin polyadenylation site and yielding the plasmid pCMVΔR8.73.

Please replace the paragraph at page 33, lines 17-26, with the following:

In another embodiment of the invention, all the HIV-derived sequences remaining in the plasmid pCMVΔR8.74 upstream of the initiating codon of the gag gene were removed, except for the consensus 5'splice donor site. At the same time, the sequence upstream of the gag gene was changed for optimal translation efficiency obtaining the plasmid pCMVΔR8.75. pCMVΔR8.75 was derived from pCMVΔR8.74 by replacing the 94 bp SstII-ClaI fragment with an SstII-ClaI oligonucleotide linker consisting of, 5'-GGGACTGGTGAGTGAATTCGAGATCTGCCGCCGCCATGGGTGCGAGAGCG TCAGTATTAAGCGGGGGAGAATTAGAT-3' (SEQ ID NO: 2) and 5'-CGATCTAATTCTCCCCCGCTTAATACTGACGCTCTCGCACCCATGGCGGCGG CAGATCTCGAATTCACTCACCAGTCCCGC-3' (SEQ ID NO: 3).

Please replace the paragraphs at page 34, lines 11-27, with the following:

The lentiviral transfer vector plasmids were derived from the plasmid pHR'-CMV-LacZ described previously in Naldini et al. Science, supra. pHR2 is a lentiviral transfer vector in which 124 bp of nef sequences upstream of the 3'LTR in pHR' were replaced with a polylinker both to reduce HIV1 sequences and to facilitate transgene cloning. pHR2 was derived from pHR'-CMV-LacZ by replacing the 4.6 kb ClaI-StuI fragment with the 828 bp ClaI-StuI fragment generated by PCR using pHR'-CMV-LacZ as the template and the oligonucleotide, 5'-CCATCGATCACGAGACTAGTCCTACGTATCCCCGGGGACGGGATCCGCGGA ATTCCGTTTAAGAC-3' (SEQ ID NO: 4) and 5'-TTATAATGTCAAGGCCTCTC-3' (SEQ ID NO: 5) in a three-part ligation with a 4.4 kb StuI-NcoI fragment and a 4.5 kb NcoI-ClaI fragment from pHR'-CMV-LacZ.

In another embodiment of the invention, pHR3 is a lentiviral transfer vector in which 148 bp of env coding sequences (including an ATG) upstream of the Rev Response Element (RRE) in pHR2 were deleted. pHR3 was derived from pHR2 by replacing the 893 bp NotI-SpeI fragment of pHR2 with a 747 bp NotI-SpeI fragment generated by PCR using pHR2 as the template with oligonucleotide primers 5'-GCGGCCGCAGGAGCTTTGTTCCTTGG-3' (SEQ ID NO: 6) and 5'-TACGTAGGACTAGTCTCG-3' (SEQ ID NO: 7).

Please replace the paragraphs at page 35, lines 1-12, with the following:

In another embodiment of the invention, pHR5 is a lentiviral transfer vector in which 310 bp gag coding sequences (all gag coding sequences downstream from amino acid 15 of the Gag protein) were deleted from pHR2. pHR5 was derived by digestion of pHR2 with NruI, addition of a NotI linker (synthetic oligonucleotide 5'-TTGCGGCCGCAA-3'; SEQ ID NO: 8), digestion with NotI to excise the 310 bp fragment, followed by religation.

In another embodiment of the invention, pHR6 is a lentiviral vector in which the 5' splice donor signal was mutated (TGGT to TGAT) to enhance production of full-length transcripts capable of being packaged. pHR6 was derived from pHR5 by replacing the 239 bp AflII-ApoI fragment with a 239 bp AflII-ApoI fragment generated by PCR using a pHR2 as the template with oligonucleotide primers 5'-CCACTGCTTAAGCCT-3' (SEQ ID NO:9) and S'--CAAAATTZ1'TGGCGTACTCATCAGTCGCCGCCCCTCG-3' (SEQ ID NO:10).

Please replace the paragraphs at page 35, line 26, to page 38, line 16, with the following:

pRRL is a lentiviral transfer vector in which the enhancer and promoter (nucleotides -233 to-1 relative to the transcriptional start site) of RSV is precisely fused to the R region of HIV-1 using an oligonucleotide linker. pRRL was derived from plasmids pRT43.RSV.F3, see W097/07225, and pHR2 by replacing the 3.4 kb EcoRI-HpaI fragment of pRT43.RSV.F3 with the .67 kb BglII-NotI fragment from pHR2 and the 1.7kb NotI-StuI fragment from pHR2 along with a synthetic EcoRI-BglII oligonucleotide linker consisting of oligonucleotides

5'-AATTGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAATAA ACGGGTCTCTCTGGTTAGACCA-3' (SEQ ID NO:11) and 5'-GATCTGGTCTAACCAGAGAGACCCGTTTATTGTATCGAGCTAGGCACTTAAA TACAATATCTCTGCAATGCGGC-3' (SEQ ID NO:12).

In another embodiment of the invention, the 5' LTR of the lentiviral vector contains the enhancer (nucleotides -233 - -50 relative to the transcriptional start site) of the Rous Sarcoma Virus (RSV) joined to the promoter region (from the position -78 bp relative to the transcriptional start site) of HIV-1 (plasmid pRLL).

pRLL is a lentiviral transfer vector in which the enhancer of RSV is fused to the promoter region of HIV-1 using an oligonucleotide linker. pRRL was derived from plasmids pRT43.RSV.F3 and pHR2 by replacing the 3.4 kb EcoRI-HpaI fragment of pRT43.RSV.F3 with the .724 kb AlwNI-NotI fragment from pHR2 and the 1.7 kb NotI-StuI fragment from pHR2 along with a synthetic EcoRI-AlwNI oligonucleotide linker consisting of the oligo, 5'-AATTGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGATC-3' (SEQ ID NO:13) and the oligonucleotide, 5'-CTGAGGGCTCGCCACTCCCCAGTCCCGCCCAGGCCACGCCTCC-3' (SEQ ID NO:14).

In another embodiment of the invention (plasmid pCCL), the 5' LTR of the lentiviral vector contains the immediate early enhancer and promoter (nucleotides -673 to -1, relative to the transcriptional start site according to Boshart et al. (Cell (1985) 41: 521-530), of human cytomegalovirus (CMV) joined to the R region of HIV-1. pCCL was derived from plasmids 5'-GATATGATCAGATC-3' (SEQ ID NO: 15) and 5'-CTGATCA-3' (SEQ ID NO: 16) and a three-part ligation along with a .54 kb AlwN-AvrII fragment and a 6.1 kb AvrII-BbsI fragment from pRRL

pRRL.SIN-45 was derived from pRRL by replacing the 493 bp BbsI-AlwNI fragment in the 3' LTR with an oligonucleotide linker consisting of synthetic oligonucleotides, 5'-GATATGATCAGAGCCCTCAGATC-3' (SEQ ID NO: 17) and

5'-CTGAGGGCTCTGATCA-3' (SEQ ID NO: 18) in a three-part ligation along with a .54 kb AlwNI-AvrII fragment and a 6.1 kb AvrII-BbsI fragment from pRRL

pRRL.SIN-78 was derived from pRRL by replacing the 493 bp BbsI-AlwNI fragment in the 3' LTR with an oligonucleotide linker consisting of, 5'-GATATGATCAGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCC TCAGATC-3' (SEQ ID NO: 19) and oligonucleotide 5'-CTGAGGGCTCGCCACTCCCCAGTCCCGCCCAGGCCACGCCTCCTGATCA-3' (SEQ ID NO: 20) in a three-part ligation along with a .54 kb AlwNI-AvrII fragment and a 6.1 kb AvrII-BbsI fragment from pRRI.

Please replace the paragraphs at page 39, line 12, to page 42, line 20, with the following:

pMDLg/p is a CMV driven expression plasmid that contains only the gag/pol coding sequences from HIV-1. First, pkat2Lg/p was constructed by ligating a 4.2 kb ClaI-EcoRI fragment from pCMVAR8.74 with a 3.3 kb EcoRI-HindIII fragment from pkat2 (Finer et al., Blood (1994) 83: 43-50) and a 0.9 kb HindIII-NcoI fragment from pkat2 along with a NcoI-ClaI DNA linker consisting of synthetic oligonucleotides 5'-CATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGAT-3' (SEQ ID NO: 21) and 5'-CGATCTAATTCTCCCCCGCTTAATACTGACGCTCTCGCACC-3' (SEQ ID NO: 22). Next, pMDLg/p was constructed by inserting the 4.25kb EcoRI fragment from pkat2Lg/p into the EcoRI site of pMD-2. pMD-2 is a derivative of pMD.G (Ory et al., supra) in which the pXF3 plasmid backbone of pMD.G has been replaced with a minimal pUC18 (Invitrogen) plasmid backbone and the 1.6 kb VSV.G encoding EcoRI fragment has been removed.

pMDLg/pRRE differs from pMDLg/p by the addition of a 374 bp RRE-containing sequence from HIV-1 (HXB2) immediately downstream of the pol coding sequences. To generate pMDLg/pRRE, the 374 bp NotI-HindII RRE-containing fragment from pHR3 was ligated into the 9.3kb NotI-BglII fragment of pVL1393 (Invitrogen) along with a HindIII-BglII DNA linker consisting of synthetic oligonucleotides 5'-AGCTTCCGCGGA-

3' (SEQ ID NO: 23) and 5'-GATCTCCGCGGA-3' (SEQ ID NO: 24) to generate pVL1393RRE (pHR3 was derived from pHR2 by the removal of HIV env coding sequences upstream of the RRE sequences in pHR2). A Not I site remains at the junction between the gag and RRE sequences. pMDLg/pRRE then was constructed by ligating the 380 bp Eco RI-SstII fragment from pV1393RRE with the 3.15 kb SstII-NdeI fragment from pMD-2FIX (pMD-2FIX is a human factor IX containing a variant of pMD-2 which has an SstII site at the 3' end of the Factor IX insert), the 2.25 kb NdeI-AvrII fragment from pMDLg/p and the 3.09 kb AvrII-EcoRI fragment from pkat2Lg/p (Finer et al., supra).

pMDLg/pRRE. 2 is a gag/pol expressing lentiviral packaging vector in which the codons for the gag amino acids 2-13 have been mutated (without changing the amino acids sequence). pMDLg/pRRE. 2 was generated by ligating an 8.4 kb ClaI-Bsu36I fragment and a 1.4 kb Bsu36I-EcoRI fragment from pMDLg/pRRE with a DNA linker consisting of synthetic oligonucleotide, 5'-aattcgagatctgccgccgccatgggagcccgggccagcgtcctgtctggaggggagctggac-3' (SEQ ID NO: 25) and 5'-cggtccagctcccctccagacaggacgctggcccgggctcccatggcggcggcagatctcg-3' (SEQ ID NO:26).

pMDLg/pRRE.3 is a gag/pol expressing lentiviral packaging vector in which the codons for the gag amino acids 2-7 have been mutated (without changing the amino acids sequence) and in which gag coding sequences for amino acids from 8 to 87 of Gag polyprotein have been deleted. Previously described experiments which were conducted to study HIV-1 MA protein functions (Reil et al., EMBO J. (1998) 17: 2699-708) demonstrated that deletion of amino acids from 8 to 87 of matrix protein (MA), which is part of Gag polyprotein, has no effect on efficiency of wild type HIV-1 entry into infected cell, when analyzed virions were pseudotyped with VSV/G. pMDLg/pRRE. 3 was generated by ligating an 6.8 kb SphI-Bsu36I fragment and a 1.4 kb Bsu36I-EcoRI fragment from pMDLg/pRRE with a 0.4kb XbaI-SphI fragment from plasmid HXB 10ACT. A8-87 described in (Reil et al., supra) and a DNA linker consisting of synthetic oligonucleotides 5' aattcgagatctgccgccgccatgggagcccgggccagcgtc-3' (SEQ ID NO: 27) and 5'-ctagagacgctggcccgggctcccatggcggcggcagatctcg-3' (SEQ ID NO: 28).

ptetMDrev is an expression vector in which HIV-1 Rev protein expression is under the control of the tet inducible tet°7/CMV hybrid promoter. The only HIV sequences contained in the vector are HXB2 rev cDNA comprising the first (nucleotides 5937 through 6045) and second (nucleotides 8379 trough 8653) exons (Genbank accession number K03455). To generate ptetMDrev, the CMV enhancer/promoter of pMD-2 was replaced with the tet°/CMV hybrid promoter from ptet/splice (Gibco/BRL), yielding ptetMD. Next, ptetMDNcoI (ATG) was generated by inserting a DNA linker consisting of synthetic oligonucleotides 5'-aattcacgcgtgccgccaccatggcaggaagaagcggagacagcgacgaagacctcctcgcggccgccagtagctgt-3' (SEQ ID NO: 29) and 5'-aattacagctactggcggccgcgaggaggtcttcgtcgctgtctccgcttcttcctgccatggtggcggcacgcgtg-3' (SEQ ID NO: 30) into EcoRI-digested ptetMD. Finally, ptetMDrev was generated by ligating a 4.6 kb AlwNI-BamHI fragment and a 615 bp Bam HI-BbsI fragment from ptetMDNcoI (ATG) with a 354bp BbsI-AlWNI fragment from pRSVrev (plasmid described in Dull et al., J Virol. (1998) 72: 8463-71).

Example 7

CONSTRUCTION OF LENTIVIRAL TRANSFER VECTORS

pHR7 is a maximally deleted lentiviral vector in which all HIV sequences between nt 43 of the gag coding sequence and the transgene have been deleted to further decrease homology between the transfer and packaging vectors. pHR7 was derived from pHR6 by ligating a 8.2kb SacII-Not I fragment and a 1.3kb XhoI-SacII fragment from pHR6 with a DNA linker consisting of synthetic oligonucleotides 5'-GGCCATTGAC-3' (SEQ ID NO: 31) and 5'-TCGAGTCAAT-3' (SEQ ID NO: 32).

pCCL7sinCMVGFPpre is a lentiviral vector which incorporates the maximally deleted 5' untranslated region of pHR7 with a self inactivating 3' LTR, a CMV 5' U3 and a post transcriptional regulatory (pre) element from the woodchuck hepatitis virus. To generate pCCL7sinCMVGFPpre, first a 329 bp AflII-XhoI fragment from pHR7 was ligated to a 1.9 kb XhoI-AvrII fragment and a 3.2 kb AvrII-AflII from pRRLsinl8hPGK.GFP to

generate pRRL7sinhPGK.GFP. Next, the hPGK internal promoter was replaced by a hCMV internal promoter by ligating a 606 bp ClaI-BamHI fragment (in which the ClaI site was "filled") from pRRLsinCMV.GFP with a 4.9 kb BamHI-AvaI fragment (in which the AvaI site was "filled") from pRRL7sinhPGK.GFP to generate pRRL7sinhCMV.GFP. Next a 600 bp SalI to EcoRI woodchuck hepatitis virus pre fragment (generated by PCR using pWHV8 (Genbank assession # J04514) as the template with primers 5'-tctagaggatccgtcgacaatcaacctctggattacaa-3' (SEQ ID NO: 33) and 5'gagctcgaattccaggcggggaggcggcccaa-3' (SEQ ID NO: 34) followed by digestion with SalI and EcoRI) was inserted into SalI and EcoRI digested pRRL7sinhCMV.GFP to generate pRRL7sinhCMV.GFPpre. Next the 704 bp AflIII to AflII fragment of pRRL7sinhCMV.GFP was replaced with the 1147 bp AflIII to AflII fragment from pCCL to generate pCCL7sinhCMV.GFPpre.

Please replace the paragraphs at page 43, line 16, to page 45, line 18, with the following:

To generate pRRLsin36PGKGFPtet 3', first a 5.6 kb Asp718 - BamHI fragment from pRRL5sinl 8PGKGFP was ligated to a 303 bp XhoI-Asp718 fragment from ptet/splice (Gibco/BRL) along with the DNA linker consisting of synthetic oligonucleotides 5'-GATCCCGGGC-3' (SEQ ID NO: 35) and 5'-TCGAGCCCGG-3' (SEQ ID NO: 36) to generate ptetINT (pRRL5sinl8PGKGFP is a vector in which the untranslated region of pRRLsinl8PGKGFP (Zufferey et. al., J. Virol., (1998) 72: 9873-9880) has been replaced with the corresponding region from pHR5) Next a 2.8 kb AflIII-Asp718 fragment from ptetINT was ligated to a 3.1 kb BclI - AflIII fragment from pRRLsin36PGKGFP (Zufferey et. al. (1998) supra) along with the DNA linker consisting of synthetic oligonucleotides 5'-GTACCCGGGTCGAGTAGGCTT-3' (SEQ ID NO: 37) and 5'-GATCAAGCCTACTCGACCCGG-3' (SEQ ID NO: 38) to generate ptet36INT. Finally a 3.4 kb BamHI-AflIII fragment from ptet36INT was ligated to a 3.6 kb AflIII-BclI fragment from pRRLsin36PGKGFP to yield pRRLsin36PGKGFPtet°3'.

pCCL7sinCMVGFPpreTet°3' is a lentiviral transfer vector maximally deleted in the 5' untranslated region, in which the 3' LTR of pCCL7sinCMVGFPpre has been replaced

with the tet-responsive 3' LTR from pRRLsin36PGKGFPtet°3'. pCCL7sinCMVGFPpreTet 3' was generated by ligating a 3.44 kb AflIII-EcoRI fragment from pCCL7sinCMVGFPpre with a 3.5 kb EcoRI-AflIII fragment from pRRLsin36PGKGFPtet°3'.

Example 9

To isolate viral RNA, 0.45 micron-pore-size (Millipore) filtered supernatants containing vector particles were adjusted for p24 content and microcentrifuged at 14,000 rpm to pellet the virions. Supernatants were aspirated and 50 μg of yeast RNA were added to each pellet as carrier. Total RNA was isolated from the samples using RNAqueous kit (Ambion) according to manufacturer instructions. DNA probe template for in vitro transcription was prepared by two cycles of PCR using a Lig'nScribe™ kit (Ambion) as instructed by the manufacturer. Probe 1 was generated by PCR using primers 5'CATCAGGCCATATCACCTAGA-3' (SEQ ID NO: 39) and 5'-GTACTAGTAGTTCCTGCTATGT-3' (SEQ ID NO: 40) and plasmid pCMVΔR8.74 to amplify a 298 bp fragment containing nucleotides 1215 through 1513 of HIV-1 HXB2 (Genbank accession number K03455). Probe 2 was generated by PCR using primers 5'-CTGCTGACATCGAGCTTGCTACA-3' (SEQ ID NO: 41) and 5'-CTAGCTCCCTGCTTGCCCATACT-3' (SEQ ID NO: 42) and plasmid pHR2 as template to amplify a 577 bp fragment containing nucleotides 336 through 913 of HIV-1 HXB2 (Genbank accession number K03455). ^{32}P antisense riboprobe then was synthesized by T7 RNA polymerase in the presence of UTP (800Ci/ml, DuPont NEN™). Full length probes were gel purified and stored in 0.5 M ammonium acetate, 1 mM EDTA, and 0.2% SDS elution buffer at-20°C. RNA protection assay was performed using a HybSpeed™ kit (Ambion) according to manufacturer instructions. rnase A/T1 mix (0.5 U/20 U per reaction, Ambion) digestion protected probe fragments were separated on 4% polyacrylamide, TBE and 8 M urea gels. For fragment size determination, ^{32}P-labeled an RNA markers were synthesized on RNA Century template set and electrophoresed in parallel. For band detection and intensity quantification, dried gels were exposed either to photofilm or a phosphorimager plate (Molecular Dynamics).

Example 10

Transfer Vector Constructs. pHR'CMV-LacZ and pHR'CMV-Luciferase have been described (Naldini et al., Science, supra). pHR2 is a lentiviral transfer vector in which the polylinker and downstream nef sequences up to the KpnI site of pHR' have been replaced with a ClaI/SpeI/SnaBI/SmaI/BamHI/SacII/EcoRI polylinker. pHR2 was generated by replacing the 3.7 kb ClaI-SacI fragment of pHR'CMVlacZ with a 607 bp ClaI-SacI fragment generated by PCR using pHR'CMVlacZ as the template with oligonucleotide primers 5'-CCATCGATGGACTAGTCCTACGTATCCCCGGGGACGGGATCCGCGGAATTCC GTTTAAGACCAATGAC-3' (SEQ ID NO: 43) and 5'-TTATAATGTCAAGGCCTCTC-3' (SEQ ID NO: 44), followed by digestion with ClaI and SacI.

Please replace the paragraphs at page 47, line 8, to page 49, line 5, with the following:

pMDLg/p is a CMV driven expression plasmid that contains only the gag/pol coding sequences from HIV-1. First, pkat2Lg/p was constructed by ligating a 4.2 kb ClaI-Eco RI fragment from pCMVAR8.74 with a 3. kb EcoRI-HindIII fragment from pkat2 (Finer et al., supra) and a 0.9 kb HindIII-NcoI fragment from pkat2 along with a NcoI-ClaI linker consisting of synthetic oligonucleotides, 5'-CATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGAT-3' (SEQ ID NO: 45) and 5'-CGATCTAATTCTCCCCCGCTTAATACTGACGCTCTCGCACC-3' (SEQ ID NO: 46). Next, pMDLg/p was constructed by inserting the 4.25kb EcoRI fragment from pkat2Lg/p into the Eco RI site of pMD-2. pMD-2 is a derivative of pMD.G (Ory et al., supra) in which the pXF3 plasmid backbone of pMD.G has been replaced with a minimal pUC plasmid backbone and the 1.6 kb VSV.G encoding EcoRI fragment has been removed.

pMDLg/pRRE differs from pMDLg/p by the addition of a 374 bp RRE-containing sequence from HIV-1 (HXB2) immediately downstream of the pol coding sequences. To generate pMDLg/pRRE, the 374 bp NotI-HindII RRE-containing fragment from pHR3 was ligated into the 9.3 kb NotI-BglII fragment of pVL1393 (Invitrogen) along with a HindIII-BglII oligonucleotide linker consisting of synthetic oligonucleotides 5'-

AGCTTCCGCGGA-3' (SEQ ID NO: 47) and 5' GATCTCCGCGGA-3' (SEQ ID NO: 48) to generate pVL1393RRE (pHR3 was derived from pHR2 by the removal of HIV env coding sequences upstream of the RRE sequences in pHR2). A Not I site remains at the junction between the gag and RRE sequences. pMDLg/pRRE was then constructed by ligating the 380 bp Eco RI-SstII fragment from pV1393RRE with the 3.15 kb SstII-NdeI fragment from pMD-2FIX (pMD-2FIX is a human factor IX containing variant of pMD-2 which has an SstII site at the 3' end of the Factor IX insert), the 2.25 kb NdeI-AvrII fragment from pMDLg/p and the 3.09 kb AvrII-EcoRI fragment from pkatlLg/p (Finer et al., supra).

Please replace the paragraph at page 57, line 23, to page 58, line 17, with the following:

In another embodiment of the invention, pRRLsin36PGKGFPtet°3' is a lentivirus vector in which the'3 LTR contains a hybrid tet°/HIV U3. The 3' U3 consists of seven copies of the tet operator (teto7) linked to the 3' 36 nucleotides of the HIV U3 including the "tata" box. pRRLsin36PGKGFPtet°3' is a conditional self inactivating (SIN) vector that, after transduction, can be activated to express full-length packagable vector transcripts only in the presence of tet-transactivator (tta) - for example, after transduction of an appropriate tta expressing packaging cell line. After transduction of any cell not expressing tta, the resulting 5' teto7/HIV U3 is essentially non-functional, even in the presence of HIV tat, significantly reducing the chance of mobilization of the vector genome. pRRLsin36PGKGFPtet°3' allows for a SIN vector which can be serially transduced ("pinged") into a tta-expressing packaging cell line to obtain a high-titer producer clone while maintaining the SIN phenotype in non-tta expressing target cells. To generate pRRLsin36PGKGFPtet°3', first a 5.6 kb Asp718-BamHI fragment from pRRL5sinl8PGKGFP was ligated to a 303 bp XhoI-Asp718 fragment from ptetsplice along with a DNA linker consisting of synthetic oligonucleotides 5'GATCCCGGGC-3' (SEQ ID NO: 49) and 5'TCGAGCCCGG-3' (SEQ ID NO: 50) to generate ptetINT (pRRL5sin18PGKGFP is a vector in which the untranslated region of pRRLsin18PGKGFP (Zufferey et al., J. Virol (1998) 72: 9873-9880) has been replaced with the corresponding region from pHR5) Next a 2.8 bk AflII-Asp718 fragment from ptetINT was ligated to a 3.1

kb BclI-AflII fragment from pRRLsin36PGKGFP (Zufferey et al. (1998) supra) along with a DNA linker consisting of synthetic oligonucleotide 5'-GTACCCGGGTCGAGTAGGCTT-3' (SEQ ID NO: 51) and 5'-GATCAAGCCTACTCGACCCGG-3' (SEQ ID NO: 52) to generate ptet36INT. Finally, a 3.4kb BamHI-AflII fragment from ptet36INT was ligated to a 3.6 kb AflII-BclI fragment from pRRLsin36PGKGFP to yield pRRLsin36PGKGFPtet°3'.